	L #	Hits	Search Text	DBs	Time Stamp
1	L1	867	galactosyltransferase$1 or galactosyl adj transferase$1	USPAT; US-PGPUB	2003/10/21 12:42
2	L2	6054	gb3 or cd77 or globotriaosylceramide	USPAT; US-PGPUB	2003/10/21 12:42
3	L3	3	2 adj synthase$1	USPAT; US-PGPUB	2003/10/21 12:43
4	L4	25	1 and 2	USPAT; US-PGPUB	2003/10/21 12:45

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	867	galactosyltransferase$1 or galactosyl adj transferase$1	USPAT; US-PGPUB	2003/10/21 12:42
2	L2	6054	gb3 or cd77 or globotriaosylceramide	USPAT; US-PGPUB	2003/10/21 12:42
3	L4	25	1 and 2	USPAT; US-PGPUB	2003/10/21 12:43
4	L3	3	2 adj synthase$1	USPAT; US-PGPUB	2003/10/21 12:43

PGPUB-DOCUMENT-NUMBER: 20030157113

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030157113 A1

TITLE: Compositions and methods for treatment of neoplastic disease

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Terman, David S.	Pebble Beach	CA	US	

APPL-NO: 09/ 751708

DATE FILED: December 28, 2000

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60173371 19991228 US

US-CL-CURRENT: 424/184.1, 435/346

ABSTRACT:

The present invention comprises compositions and methods for treating a tumor or neoplastic disease in a host, The methods employ conjugates comprising superantigen polypeptides, nucleic acids with other structures that preferentially bind to tumor cells and are capable of inducing apoptosis. Also provided are superantigen-glycolipid conjugates and vesicles that are loaded onto antigen presenting cells to activate both T cells and NKT cells. Cell-based vaccines comprise tumor cells engineered to express a superantigen along with glycolipids products which, when expressed, render the cells capable of eliciting an effective anti-tumor immune response in a mammal into which these cells are introduced. Included among these compositions are tumor cells, hybrid cells of tumor cells and accessory cells, preferably dendritic cells. Also provided are tumoricidal T cells and NKT cells devoid of inhibitory receptors or inhibitory signaling motifs which are hyperresponsive to the the above compositions and lipid-based tumor associated antigens that can be administered for adoptive immunotherapy of cancer and infectious diseases.

---------- KWIC ---------

Detail Description Paragraph - DETX (164):

[0211] The synthetic pathway for neutral glycosphingolipids in eukaryotic cells is known. Glucosylceramide (GlcCer) is the precursor of lactosylceramide

(LacCer), which leads, in order, to Gb3 and globotetraosylceramide (Gb4). Different Golgi enzymes are responsible for addition of monosaccharides from nucleotide-sugar donors in each step of the pathway. **Globotriaosylceramide synthase** (UDP-galactose:lactosylcerami- de a1-4-galactosyltransferase) has been purified. In the cytoplasm, the a-subunit of the Shiga toxin or VT is processed by a trypsin-like cleavage. The "activated" 27-kDa a-subunit inactivates 60S ribosomes by depurination of a single nucleotide in 28S rRNA, rendering ribosomes incapable of carrying out peptide elongation.

PGPUB-DOCUMENT-NUMBER: 20030138807

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138807 A1

TITLE: UDP-galactose: beta-D-galactose-R 4-alpha-D-galac-tosyltransferase, alpha4Gal-T1

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Clausen, Henrik	Holte		DK	
Steffensen, Rudi	Pandrup		DK	
Bennett, Eric Paul	Lyngby		DK	

APPL-NO: 10/ 217335

DATE FILED: August 8, 2002

RELATED-US-APPL-DATA:

child 10217335 A1 20020808

parent continuation-of PCT/DK01/00087 20010209 US UNKNOWN

non-provisional-of-provisional 60182037 20000211 US

US-CL-CURRENT: 435/6, 435/193 , 435/320.1 , 435/325 , 435/69.1 , 435/7.1 , 530/388.26 , 536/23.2

ABSTRACT:

A novel gene defining a novel enzyme UDP-Galactose: .beta.-D-Galactose-R 4-.alpha.-D-galactosyltransferase, termed .alpha.4Gal-T1, with unique enzymatic properties is disclosed. The invention discloses isolated DNA molecules and DNA constructs encoding .alpha.4Gal-T1 and derivatives thereof by way of amino acid deletion, substitution or insertion exhibiting .alpha.4Gal-T1 activity, as well as cloning and expression vectors including such DNA, cells transfected with vectors, and recombinant methods for providing .alpha.4Gal-T1. The enzyme .alpha.4Gal-T1 and .alpha.4Gal-active derivatives thereof are disclosed. Further, the invention discloses methods of obtaining .alpha.1, 4galactosyl glycosylated glycosphingolipids by use of an enzymatically active .alpha.4Gal-T1 protein thereof or by using cells stably transfected with a vector including DNA encoding an enzymatically active .alpha.4Gal-T1 protein as an experession system for recombinant production of such glycosphingolipids. Also a method for the identification of DNA sequence variations in the .alpha.4Gal-T1-coding exon by PCR, and detecting the presence of DNA sequence variation, are disclosed.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (2):

[0001] The present invention relates generally to the biosynthesis of glycans found as free oligosaccharides or covalently bound to proteins and glycosphingolipids. This invention is more particularly related to nucleic acids encoding an UDP-D-galactose: .beta.-D-galactose-R 4-.alpha.-D-galactosyltransferase (.alpha.4Gal-transferase), which add galactose to the hydroxy group at carbon 4 of D-galactose (Gal). This invention is more particularly related to a gene encoding the blood group P.sup.k **(Gb3) synthase,** termed .alpha.4Gal-T1, probes to the DNA encoding .alpha.4Gal-T1, DNA constructs comprising DNA encoding .alpha.4Gal-T1, recombinant plasmids and recombinant methods for producing .alpha.4Gal-T1, recombinant methods for stably transfecting cells for expression of .alpha.4Gal-T1, and methods for identification of DNA polymorphism in patients.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	867	galactosyltransferase$1 or galactosyl adj transferase$1	USPAT; US-PGPUB	2003/10/21 12:42
2	L2	6054	gb3 or cd77 or globotriaosylceramide	USPAT; US-PGPUB	2003/10/21 12:42
3	L3	3	2 adj synthase$1	USPAT; US-PGPUB	2003/10/21 12:43
4	L4	25	1 and 2	USPAT; US-PGPUB	2003/10/21 12:45

PGPUB-DOCUMENT-NUMBER: 20030157113

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030157113 A1

TITLE: Compositions and methods for treatment of neoplastic disease

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Terman, David S.	Pebble Beach	CA	US	

APPL-NO: 09/ 751708

DATE FILED: December 28, 2000

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60173371 19991228 US

US-CL-CURRENT: 424/184.1, 435/346

ABSTRACT:

The present invention comprises compositions and methods for treating a tumor or neoplastic disease in a host, The methods employ conjugates comprising superantigen polypeptides, nucleic acids with other structures that preferentially bind to tumor cells and are capable of inducing apoptosis. Also provided are superantigen-glycolipid conjugates and vesicles that are loaded onto antigen presenting cells to activate both T cells and NKT cells.
Cell-based vaccines comprise tumor cells engineered to express a superantigen along with glycolipids products which, when expressed, render the cells capable of eliciting an effective anti-tumor immune response in a mammal into which these cells are introduced. Included among these compositions are tumor cells, hybrid cells of tumor cells and accessory cells, preferably dendritic cells.
Also provided are tumoricidal T cells and NKT cells devoid of inhibitory receptors or inhibitory signaling motifs which are hyperresponsive to the the above compositions and lipid-based tumor associated antigens that can be administered for adoptive immunotherapy of cancer and infectious diseases.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (40):
[0038] For in vivo immunization, tumor cells are transfected with nucleic acids encoding SAgs together with a carbohydrate modifying enzyme such as **galactosyl transferase** to produce the Gal epitope, Staphylococcal

hyaluronidase, Streptococcal capsular polysaccharide, Staphylococcal erythrogenic toxin, Staphylococcal Protein A, Staphylococcal b hemolysin, Staphylococcal coagulase, costimulants such as B7-1 and B7.2, chemoattractants and chemokines. SAgs are also cotransfected into tumor cells with gene clusters encoding the biosynthesis of highly immunogenic microbial Lipid A, membrane or capsular polysaccharides, lipoproteins and peptidoglycans. Nucleic acids are useful when transfected alone. However combinations are preferred. The cotransfection into tumor cells of the SAg-encoding nucleic acid together with the nucleic acids encoding Gal or GalCer biosynthesis is particularly useful. The cotransfection into tumor cells of the nucleic acid encoding SAg with nucleic acids encoding Staphylococcal erythrogenic toxins and hyaluronidase allows the transfected tumor cells to simulate the in vivo inflammatory activity of a Staphylococcus or leukocyte or macrophage by secreting enzymes and toxins which induce a sterile cellulitis in tumor sites.

Detail Description Paragraph - DETX (51):
[0099] h. Conjugates of SAg-(Gb2 or **Gb3** or Gb4)

Detail Description Paragraph - DETX (52):
[0100] i. Conjugates of SAg-(Gb2 or **Gb3** or Gb4)-CD1

Detail Description Paragraph - DETX (53):
[0101] j. GPI anchored conjugates: SAg-GPI-(Gb2 or **Gb3** or Gb4)

Detail Description Paragraph - DETX (54):
[0102] l. GPI anchored conjugates: SAg-GPI-(Gb2 or **Gb3** or Gb4)-CD1

Detail Description Paragraph - DETX (103):
[0150] Cells transfected with nucleic acid encoding a SAg may also express a tumor associated antigen that is potentially present on host cancer cells. For example, nucleic acid encoding a known tumor antigen are transfected into the SAg-containing cell, or a tumor cell that endogenously contains many different tumor antigens are transfected with SAg-encoding nucleic acid. In the latter case, additional nucleic acids encoding other polypeptides are transfected into the tumor cell. For example, nucleic acid encoding a carbohydrate modifying enzyme such as .alpha. 1,3-**galactosyltransferase,** adhesion molecule, costimulatory molecule such as B7-1 and B7-2, MHC class I molecule and/or MHC class II molecule are cotransfected into tumor cells together with nucleic acid encoding a SAg. SAg-encoding nucleic acid can encode a mutant, variant, and/or modified form of a SAg. These forms can be used to transfect T cells, alone or in combination with wild-type SAg-encoding acid.

Detail Description Paragraph - DETX (118):
[0165] Cells are transfected in vivo or in vitro. When transfected in vivo, the cells are of host origin. When transfected in vitro, the cells are autologous, allogeneic, or xenogeneic to the host to provide additional immunogenicity. In addition to being transfected with nucleic acid encoding a

SAg, the cells are transfected with nucleic acid encoding any other polypeptide including, without limitation, .alpha.-galactosyltransferase, staphylococcal hyaluronidase and/or erythrogenic toxin, streptococcal capsular polysaccharide, CD44, tumor antigen, costimulatory molecule such as B7-1 and B7-2, adhesion molecules, MHC class I molecule and/or MHC class II molecule. Nucleic acids encoding the molecules are cotransfected with the SAgs. But for others, including but not limited to Staphylococcal hyaluronidase, erythrogenic toxin, Streptococcal capsular polysaccharide and CD44 genes, the nucleic acids encoding the SAgs are fused to other nucleic acids resulting in expression of a fusion protein. Methods for in vivo and in vitro transfection of cells are well known. For example, two books in the series Methods in Molecular Medicine published by Humana Press, Totowa, N.J., describe in vivo and in vitro transfection protocols that are adaptable to the present invention (Vaccine Protocols edited by Robinson et al., (1996) in Gene Therapy Protocols edited by Robbins et al., Humana Press, Totowa, N.J. (1997)). Transfection protocols are also discussed elsewhere ((Sambrook, J. et al., Molecular Cloning, Second Edition, Cold Springs Harbor Laboratory Press, Plainview, N.Y., (1989)). In addition, use of various vectors to target epithelial cells, use of liposomal constructs, methods of transferring nucleic acid directly into T cells, hematopoietic stem cells, and fibroblasts, methods of particle-mediated nucleic acid transfer to skin cells, and methods of liposome-mediated nucleic acid transfer to tumor cells have been described elsewhere. (Felgner, P L et al., Cationic Lipids for Intracellular Delivery of Biologically Active Molecules, U.S. Pat. No. 5,459,127, issued Oct. 17, 1995; Felgner, P L, Cationic Lipids for Intracellular Delivery of Biologically Active Molecules, U.S. Pat. No. 5,264,618, issued Nov. 23, 1993; Felgner, P L, Exogenous DNA Sequences in a Mammal, U.S. Pat. No. 5,580,859 issued Dec. 3, 1996; Felgner, P L, A Protective Immune Response in a Mammal by Injecting a DNA Sequence, U.S. Pat. No. 5,589,466 issued Dec. 31, 1996).

Detail Description Paragraph - DETX (163):

[0210] SAgs have an affinity for glycosphingolipids especially those with terminal or subterminal Gal(.alpha.1-4)Gal residues. Such residues are expressed on tumor cells as Gal(.alpha.1-4)Gal(.beta.1-4)GlcCeramide (**globotriaosylceramide or Gb3**) and Gal(.alpha.1-4)GalCeramide (galabiosylceramide or Gb2). **Gb3** and Gb2 also known as **CD77,** Burkitt's lymphoma antigen, and the human blood group p.sup.k antigen are the natural receptors for Shiga toxins and VT's . Shiga toxin, a 69-kDa complex of proteins comprised of five b-subunits (7 kDa each) and one a-subunit (30 kDa) has high affinity for the terminal digalactose of **Gb3** or Gb2. Methods for their preparation and isolation are described in Example 41. Once bound to the tumor cell, these toxins are internalized and induce apoptosis.

Detail Description Paragraph - DETX (164):

[0211] The synthetic pathway for neutral glycosphingolipids in eukaryotic cells is known. Glucosylceramide (GlcCer) is the precursor of lactosylceramide (LacCer), which leads, in order, to **Gb3** and globotetraosylceramide (Gb4). Different Golgi enzymes are responsible for addition of monosaccharides from nucleotide-sugar donors in each step of the pathway. **Globotriaosylceramide** synthase (UDP-galactose:lactosylcerami- de a1-4-**galactosyltransferase**) has been purified. In the cytoplasm, the a-subunit of the Shiga toxin or VT is

processed by a trypsin-like cleavage. The "activated" 27-kDa a-subunit inactivates 60S ribosomes by depurination of a single nucleotide in 28S rRNA, rendering ribosomes incapable of carrying out peptide elongation.

Detail Description Paragraph - DETX (165):

[0212] The present invention provides therapeutically active soluble complexes comprising SAg and glycosphingolipids which have terminal or subterminal Gal(.alpha. 1-4)Gal residues and Shiga toxin receptors **Gb3** and Gb2, (collectively referred to as "GTSG1-4"). These complexes include but are not limited to SAg -GPI-GTSG1-4 complexes, and synthetic and functional derivatives thereof. Such structures appear naturally on surfaces of certain tumor cells such as astrocytoma, Burkitt's lymphoma and ovarian carcinoma. Methods of preparing and isolating glycosylceramides and VTs are given in Examples 41 and 55. SAgs also have a demonstrable affinity for galactosylceramides containing Gal(.alpha. 1-4)Gal residues. Methods of assessing SAg binding to GTSG1-4 are provided given in Example 43. These conjugates are also shed from SAg-transfected tumor cells as binary complexes of SAg-GTSG1-4 or ternary complexes of SAg-GPI-GTSG1-4, in free form, as vesicles or as exosomes(see Sections 38 and Example 38). Methods of isolating and characterizing these shed complexes appear in Section 38 and Example 42. The complexes may also be prepared by chemical or genetic methods (Example 5). SAg-GTSG1-4 or SAg-GPI-GTSG1-4 complexes or exosomes are useful as a preventative vaccine or against established tumor. They are also useful in vivo by direct administration or ex vivo where they are loaded onto antigen presenting cells comprising CD1 or MHC receptors to activate NKT and T cells to produce tumor specific effector T or NKT cells for adoptive therapy of cancer (Examples 5, 7, 14, 15, 16, 18-23, 38).

Detail Description Paragraph - DETX (167):

[0214] Additional immunogenic complexes comprising SAgs bound to tumor cells, DCs DC/tc constructs expressing surface Gb2 and **Gb3** or other glycosphingolipids containing terminal Gal(.alpha. 1-4)Gal are prepared by transfecting these cells with nucleic acids encoding a SAg. The transfected cell expresses the SAg in the context of the glycosphingolipid comprising the terminal or subterminal Gal(.alpha. 1-4))Gal moiety. Alternatively, free or GPI linked glycolipids containing SAg peptides or polypeptides bind to tumor cells or accessory cells in tissue culture (Section 38). The expression of **Gb3** and Gb2 on tumor cells is optionally upregulated by various cytokines, including IFN.alpha. and TNFa, before contacting the SAg

Detail Description Paragraph - DETX (171):

[0218] The present invention contemplates the induction of apoptosis in tumor cells expressing Gb2 and **Gb3** (or other glycosphingolipids containing terminal Gal(.alpha. 1-4)Gal) by using free SAgs, conjugates and fused DNA that comprises SAg, SAg peptide or SAg-encoding DNA fused to intact VT or to VT A or B chains. Preparation of these conjugates and fusion proteins from their corresponding DNA, polypeptides or functional derivatives is provided in Examples 1 and 5. These conjugates induce apoptosis by binding to tumor cell glycosphingolipid receptors having terminal Gal(.alpha. 1-4)Gal. Methods of assessing tumor cell apoptosis are in Example 44. CD19 or IFN.alpha. peptide

sequences and generic carbohydrate recognition domains which bind Gal(.alpha. 1-4)Gal structures are also useful. CD19, a B-cell restricted differentiation antigen, naturally binds to **Gb3** and Gb2 on the cell surface which incudes apoptosis. CD19 has VT-like sequences in the N-terminal extracellular domain (NBRF protein data bank) that have 41%, 34% and 37% sequence identity to VT1, VT2, and VT2e B subunits, respectively. When compared to a consensus VT B sequence, the CD19 sequences show 49% identity. Binding of these peptide sequences to membrane-Gal(.alpha. 1-4)Gal containing glycolipids facilitates receptor mediated induction of apoptosis.

Detail Description Paragraph - DETX (172):

[0219] The IFN.alpha. receptor has a 63-kDa extracellular peptide with regions of amino acid identity to domains in the VT B subunit implicated as Gb2/**Gb3** binding sites.

Detail Description Paragraph - DETX (173):

[0220] The preferred targets of the above conjugates on tumor cells are the naturally expressed Shiga toxin receptors **Gb3** and Gb2 with a terminal-Gal(.alpha. 1-4)Gal. Astrocytomas and Burkitt's lymphomas are the preferred tumors as they naturally express glycosphingolipid receptors. However, any tumor expressing the appropriate receptor is appropriate. Tumor cells which express either engineered or natural functional derivatives, or mutants of these glycosphingolipid receptors, are also useful. Receptor expression on the target cells is optionally upregulated by cytokines such as IFN.gamma. and TNF.alpha.. Tumor cell sensitivity to the cytotoxic effects of a VT is enhanced by administration of interleukin-1b before the addition of the conjugates. Tumor cells which do not naturally display **Gb3** or Gb2 acquire these structures by transfer from free, soluble structures or liposomes which express the missing glycosphingolipid receptor (Section 38, Example 5). The reconstituted tumor cells bearing the appropriate glycolipid receptors are thus targeted for apoptosis by the above constructs and conjugates.

Detail Description Paragraph - DETX (184):

[0231] In a fifth approach, the **-galactosyltransferase** is transfected into Fabry's disease cells, thereby adding to the usual accumulation due to the catabolic enzyme deficiency. Such cells should have massive accumulations of -galactosylceramides.

Detail Description Paragraph - DETX (198):

[0245] The .alpha.Gal epitope is expressed by endothelial cells in xenografts such as pig organs is a major antigenic target causing hyperacute organ rejection in human transplant patients. This hyperacute rejection appears to involve a complement dependent mechanism that occurs within a few minutes. An .alpha.1-3-**galactosyltransferase** is an enzyme capable of producing .alpha. 1-3-galactose-.beta.1-4-N-acetylglucosamine moiety by adding a terminal galactose residue to a subterminal galactose residue via an a1-3 linkage. In addition, the .alpha. 1-3-**galactosyltransferase** is not expressed by human and certain primate cells. Humans contain xenoreactive natural antibodies that recognize .alpha.Gal. For example, anti-Gal antibodies bind to

pig endothelial cells that express the Gal epitope. These anti-Gal antibodies are naturally occurring IgM antibodies recently found to be present in large amounts in human serum. Surface expression of the .alpha.Gal epitope on tumor cells is achieved by transfecting a cell with a cDNA clone encoding the .alpha.1-3-**galactosyltransferase**. While tumor cells are the preferred cells for transfection, other cells such as accessory cells or immunocytes are also contemplated as being within the scope of this invention.

Detail Description Paragraph - DETX (199):

[0246] Nucleic acids encoding .alpha.1-3-**galactosyltransferase** polypeptides are known (Sandrin, M S et al., Proc. Natl. Acd. Sci. USA 90: 11391-11395 (1993)). A cDNA clone encoding murine 1-3-**galactosyltransferase** is prepared using the known sequence of this protein and the polymerase chain reaction (PCR) technique (Dabrowski, P L et al., Transplant. Proc. 26: 1335-1337 (1994). Briefly, two oligonucleotide primers are synthesized: 5'-GAATTCAAGCTTATGATCACTATGCTTCA- AG-3', which is a sense primer that encodes the first 6 amino acids of the mature .alpha.1-3-**galactosyltransferase** and contains an HindIII restriction site; and 5'-GAATTCCTGCAGTCAGACATTATTCTAAC-3', which is an anti-sense primer that encodes the last 5 amino acids of the premature 1-3-**galactosyltransferase** and contains an in-frame termination codon and PstI restriction site. These primers amplify a 1185 bp fragment from a C57BL/6 spleen cell cDNA library that is subsequently purified, digested with HindIII and PstI (Pharmacia LKB) restriction endonucleases, and directionally cloned into HindIII/Pst I-digested expression vector such as CDM8 vector. After verifying the correct sequence, the .alpha.1-3-**galactosyltransferase**-containing expression vector is transfected into heterologous cells such as COS cells to confirm activity. Activity can be confirmed by testing transfected cells for Gal expression using the IB4 lectin (Sigma) of Griffonia simplicifolia that binds to .alpha.Gal residues.

Detail Description Paragraph - DETX (200):

[0247] In the preferred mode, cells transfected with nucleic acids encoding a SAg are co-transfected with nucleic acids that encode an **-galactosyltransferase**. Alternatively, nucleic acids encoding the transferase are transfected into a separate cell population which is coadministered with the SAg transfected cell population.

Detail Description Paragraph - DETX (201):

[0248] The SAg-encoding nucleic acid can be transfected into cells which already express Gal epitope. In addition, any cell can be transfected with the **-galactosyltransferase**-encoding nucleic acid. For example, .alpha.Gal-negative human tumor cells or tumor cell lines such as melanoma or adenocarcinoma are transfected with nucleic acid encoding the **-galactosyltransferase**. Tumor cells transfected with **-galactosyltransferase**-encoding nucleic acid express the .alpha.Gal on their surface and are rapidly rejected when administered to a host with preexisting .alpha.Gal specific antibodies. Methods of transfection are given in Example 1.

Detail Description Paragraph - DETX (203):

[0250] The ability of .alpha.Gal-transfected tumor cells to induce rejection is demonstrated by implantation into severely compromised immune deficient (SCID) mice that have been reconstituted with human T and B cells and transfused with normal human plasma containing the naturally occurring human antibodies specific for the .alpha.Gal epitope. In this case, tumor cells transfected with **-galactosyltransferase**-encodin- g nucleic acid is rejected while untransfected cells are not. Similarly, tumor cells transfected with **-galactosyltransferase**-encoding nucleic acid is rejected when implanted into species such as humans which synthesize antibodies to the .alpha.Gal epitope compared to untransfected control tumor cells that are unaffected by the treatment.

Detail Description Paragraph - DETX (204):

[0251] For example, pretreatment with 10.sup.5-10.sup.7**-galactosyltransferase** transfected tumor cells subcutaneously followed by implantation of untransfected tumor cells prevents the outgrowth of untransfected malignant tumor cells. Hence, the **-galactosyltransferase** transfected tumor cells function as a vaccine. Further, **-galactosyltransferase** transfected cells implanted into animals after untransfected tumors are established induce rejection of an established untransfected tumor.

Detail Description Paragraph - DETX (205):

[0252] To test for the presence of .alpha.Gal on a cell surface, .alpha.1-3 **galactosyltransferase** knockout mice that do not express the Gal antigen are used. The .alpha.1-3 **galactosyltransferase** knockout mice are described elsewhere (Tearle et al., Transplantation 61:13-19 (1996) and Shinkel et al., Transplantation 64:197-204 (1997)). A syngeneic tumor cell that is .alpha.Gal negative such as B16 melanoma variants is transfected with nucleic acids that encode a given carbohydrate modifying enzyme. These transfected cells are then implanted into the knockout mouse that received plasma containing .alpha.Gal specific antibodies. Tumors do not grow in animals containing Gal specific antibodies if the Gal epitope is expressed. Thus, hosts implanted with Gal positive tumor cells exhibit less growth than those exhibited in hosts implanted with tumor cells that are Gal negative.

Detail Description Paragraph - DETX (206):

[0253] .alpha.Gal negative transgenic animals are prepared which are useful for testing .alpha.Gal expressing tumors. To produce these animals, nucleic acids encoding .alpha.Gal fucosyltransferase are transfected into .alpha.Gal positive mice. The fucosyltransferase dominates the usage of substrate N-acetyllactosamine and precludes **-galactosyltransferase** from utilizing this substrate. The transgenic mice do not express--.alpha.Gal on the cell surface. In this way, transgenic mice with the H antigen rather than the .alpha.Gal antigen develop. Transgenic guinea pigs producing minimal .alpha.Gal are also created in this way. These animals are used as models for testing their capacity to reject syngeneic .alpha.Gal positive tumors. These systems also permit the testing of .alpha.Gal specific antibodies for anti-tumor effects after they are passively infused into animals bearing .alpha.Gal positive

tumors.

Detail Description Paragraph - DETX (209):
[0256] Alternatively, cells expressing the B antigen or selectin antigen are transfected with **-galactosyltransferase**-encoding nucleic acid that competes successfully with fucosyltransferases for N-acetyl-lactosamine substrate and preferentially expresses the .alpha.Gal epitope

Detail Description Paragraph - DETX (211):
[0258] Co-transfection of tumor cells with nucleic acid encoding SAg and nucleic acid encoding a **galactosyltransferase,** sialidase, and/or glycosyltransferase results in expression of SAg, GalCer, .alpha.Gal, or other glycolipids on the cell surface. These tumor cells are used to stimulate T or NKT cells ex vivo to produce a population of tumor specific effector cells which are deployed for adoptive immunotherapy of cancer.

Detail Description Paragraph - DETX (265):
[0312] The Shiga toxin of Shigella dysenteriae and Shiga-like toxins of E. coli (Verotoxins) are a family of related toxins which have similar amino acid sequences and biological activities. The A subunit of Shiga toxin has a molecular mass of 31 kDa which associates with five to the 7 kDa B subunits. The A subunits is proteolytically cleaved into A1 and A2. It is the A1 fragment which is biologically active. The host cell receptor for Shiga toxin is the glycolipid Gal(.alpha.1-4)Gal(.beta.1-4) GlcCeramide (globotriosylceramide; **Gb3**) and for Shiga-like toxin I (SLTI) and SLTII of E. coli is Gal(.alpha.1-3)GalCeramide (Galabiosylceramide). The binding specificity is dependent on both sugars residues and the lipid moiety. The Shiga toxin is known to inhibit protein synthesis. It is a RNA N-glycosidase enzyme whose site of action is the 60S ribosomal subunit. The toxins remove an adenine base from position 4324 on the aminoacyl-transfer RNA binding site of 28S ribosomal RNA hence preventing peptide length elongation. The effect on protein synthesis is similar to that of diphtheria toxin and Pseudomonas aeruginosa exotoxin A. The SLTI and II toxins of E. coli and encoded by lysogenic phage. Its expression is controlled by iron concentration in the growth medium by way of the fur gene and iron box repressor protein binding site. Clostridia difficile toxins A and B also bind to anomeric galactose epitopes on cell membranes and induce membrane associated enzymes and inhibit G protein activation which results in cell death. Tumor cells transfected with a **galactosyltransferase** genes to produce the .alpha.-Gal epitope are a susceptible to lysis by both the Shiga-like toxins and C. difficile toxin. The expression of the .alpha.-Gal epitope is enabled by the transfection of nucleic acids encoding .alpha.-Gal-transferase into tumor cells.

Detail Description Paragraph - DETX (335):
[0382] Phage display is preferably done using the filamentous phage f88-4 and comprises forming a fusion that results in the C terminus of the "selected" (i.e., inserted gene's) product and the N terminus of the phage protein gVIIp. Peptides of various enterotoxins are expressed in the phage display--most preferably peptides that bind to the SAg receptor on colon carcinoma cells.

These peptides retain their capacity to bind to the TCR and to activate T cells. Also contemplated within this invention is phage display of SAg plus nucleic acid encoding synthesis of GalCer and/or the Gal epitope. DNA for synthesis of GalCer is preferably isolated from Sphingomonas paucimobilis; DNA encoding the **galactosyl transferase** for synthesis of Gal is preferably isolated from Klebsiella aerobacter, Serratia, E. coli and Salmonella organisms which naturally a produce and express these epitopes. The phage displays are administered in vivo and are capable of initiating a potent immune response to the tumor using the protocols described in Examples 5 and 13 and Section 19, above. These preparations are also useful for activating T cells or NKT cells ex vivo to produce a tumor specific effector cells for use in adoptive immunotherapy (Examples 2-5, 14-16, 18-23).

Detail Description Paragraph - DETX (336):

[0383] Viral infection of a host cell having the **galactosyl transferase** results in the shedding of virions that express the Gal epitope. When a host mammalian cell has been transfected with nucleic acid encoding SAg, the virus can coexpress the .alpha.-Gal epitope and the SAg on its surface. Such a viral construct is administered in vivo to achieve a therapeutic effect, or, in another embodiment, is employed ex vivo to produce tumor specific effector T or NKT cells for use in adoptive immunotherapy of cancer (Examples 2, 3, 7, 15, 16, 18-23).

Detail Description Paragraph - DETX (470):

[0517] The present invention also includes the additional introduction, into the S/D/t cell of with additional nucleic acids. In one embodiment, the additional nucleic acid encodes the particular **galactosyltransferase** enzyme that catalyze the synthesis of the "heterograft epitope" .alpha. Gal. In another embodiment, the additional nucleic acid encodes enzymes that synthesize galactosylceramide which is the "natural" epitope recognized by the invariant chain of NKT cells.

Detail Description Paragraph - DETX (504):

[0551] DNA encoding the **galactosyltransferase** that synthesizes the saccharide structure containing the a .alpha. Gal epitope, and gene clusters encoding the biosynthetic pathway for LPS are described in Schnaitman C A, et al., Microbiol. Rev. 57: 655-682 (1993). DNA is extracted from bacteria which biosynthesize these molecules and used to transfect DCs, tumor cells, or S/D/t cells For creation of the GalCer structure, the source of DNA is Sphingomonas paucimobilis organisms. Nucleic acids encoding the pathways for biosynthesis of .beta.-1,3-glucans, peptidoglycans, and protein A have been cloned from insects and Staphylococcus aureus, respectively. These nucleic acids are cloned into suitable expression vectors and introduced into the target cells. Resulting S/D/t cells thus express SAg as well as the anti-tumor motif structure.

Detail Description Paragraph - DETX (910):

[0954] Viral DNA, nucleic acid expression cassettes or plasmids or bacteriophages encoding the constructs given in Table II may be used for in

vivo immunization in place of naked DNA. Viruses may also acquire the .alpha.-Gal epitope after transfection into tumor cells which contain the a-**galactosyltransferase** enzyme either naturally or via transfection. The virus must possess the intact N-acetyllactosamine substrate for the **galactosyl-transferase** in order to express the .alpha.-Gal. The viruses shedding from these cells will express the aGal epitope. The virus also contains peptide sequences for SAg and tumor associated antigen acquired from the tumor cells which were previously transfected with nucleic acids encoding SAg and tumor antigen. The shed virus may also express staphylococcal or streptococcal hyaluronidase and capsular polysaccharide sequences obtained from host tumor cell or accessory cells previously transfected with nucleic acids encoding these genes. The shed virus expressing .alpha.-Gal, SAg, hyaluronidase and capsular polysaccharide is capable of initiating a potent tumoricidal response when administered to hosts with established tumors or when used as a tumor vaccine against potential tumors.

Detail Description Paragraph - DETX (983):

[1025] To prepare glycolipid, phytosphingosine, apolipoprotein, oxyLDL or receptor containing liposomes, 400 mg of galabiosylceramide (Gb2) globotriosylceramide (**Gb3**), globotetraosylceramide (Gb4), galactosylceramide (GalCer),glucosylceramide (GlcCer), phytoshpingosine, oxyLDL or apolipoprotein are dried with 200 mg of phosphatidylethanolamin- e (PE) and 200 mg of phosphatidylserine (PS) under a stream of nitrogen gas. 400 ml of sterile isotonic PBS, pH 7.4, is added to the lipid, and the mixture is sonicated using a water bath sonicator for 30 minutes. Liposome preparations are used immediately.

Detail Description Paragraph - DETX (1592):

[1598] Globotrioslceramides (**GB3**) and globotetraosylceramide (Gb4) are purified from human renal tissue. Briefly, the chloroform/methanol tissue extract is first applied on a Bio-Sil A (Bio-Rad) silica column in chloroform. The column is extensively washed with chloroform, and neutral glycolipids are eluted with acetone/methanol,9:1 (vol/vol). The neutral glycolipid fraction is then applied on a second Bio-Sil A column in chloroform/methanol, 98:2 (vol/vol). Glycolipids are then resolved with a linear solvent gradient comprising equal weights of chloroform/methanol 15:1 (vol/vol), to chloroform/methanol, 4:1 (vol/vol). Galabiosylceramide (Gb2) or Gal(.alpha.1-4)Gal ceramide from marine sponge may be obtained, for example, from Dr T. Matsubara (Department of Chemistry, Kinki University, Kowakae. Japan).

Detail Description Table CWU - DETL (8):

8TABLE V Ex vivo Modes of Antigen Presentation to T Cells or NKT Cells to Produce Tumor Specific Effector Cells A. Tumor Cells, Accessory Cells, Accessory Cell/Tumor Cell Hybrids, e.g., DC/Tumor Cell) Transfected with: 1. SAg-encoding nucleic acid ·2. SAg-encoding nucleic acid and tumor associated antigen nucleic acids (to include arrays of tumor associated epitopes) 3. SAg nucleic acid and MHC class I or II nucleic acids. 4. SAg-encoding nucleic acid and co-stimulatory nucleic acids. 5. SAg-encoding nucleic acid and adhesion molecule nucleic acids. 6. SAg-encoding nucleic acid and

a-**galactosyltransferase** synthetic nucleic acids or xenogeneic species specific nucleic acids. 7. SAg-encoding nucleic acid and chemoattractant nucleic acids 8. SAg-encoding nucleic acid and glycosylceramide synthesis nucleic acids 9. SAg nucleic acid and lipopolysaccharide synthesis nucleic acids 10. SAg-encoding nucleic acid and microbial lipoprotein or polysaccharide or peptidoglycan membrane or capsular synthesis nucleic acids 11. SAg-encoding nucleic acid and SAg receptor nucleic acids 12. SAg-encoding nucleic acid and CD1 receptor synthesis nucleic acids 13. SAg-encoding nucleic acid and CD14 receptor synthesis nucleic acids 14. SAg-encoding nucleic acid and SAg promoter and/or global regulator nucleic acids 15. SAg-encoding nucleic acid and oncogene and/or transcription factor nucleic acids 16. SAg-encoding nucleic acid and angiogenesis factor or receptor nucleic acids 17. SAg-encoding nucleic acid and growth factor receptor nucleic acids 18. SAg-encoding nucleic acid and cell cycle protein nucleic acids 19. SAg-encoding nucleic acid and heat shock protein nucleic acids 20. SAg-encoding nucleic acid and chemokine nucleic acids 21. SAg-encoding nucleic acid and cytokine nucleic acids 22. SAg-encoding nucleic acid and tumor suppressor nucleic acids 23. SAg-encoding nucleic acid and antigen processing and trafficking nucleic acids B. Additional in vitro Stimulatory Agents (preferred receptor) 1. Tumor peptides (Class I or Class II) 2. Tumor peptide-SAg conjugates or fusion proteins (Class I or Class II). 3. Lipopolysaccharide-SAg conjugate (Class II or CD14) a. arabinose b. mycolic acid c. teichoic acid d. muramic acid (Staphylococcal cell wall glycoprotein) e. mannan proteoglycans f. chondroitin-sulfate 4. Glycosylated SAgs. (Class II or mannose) 5. SAg-glycosylceramide conjugates (class II or CD1) a. GalCer conjugate b. Gal conjugate 6. SAg-proteosome conjugates 7. SAg or glycosylated SAg or SAg-glycosylceramide conjugates or SAg-lipopolysaccharide or SAg- peptidoglycan conjugates coupled to proteosomes 8. SAg or glycosylated SAg or SAg-glycosylceramide conjugates or SAg-lipopolysaccharide conjugates or SAg-peptidoglycan conjugates expressed on or coupled to liposomes 9. Conjugates having having a Superantigen component (polypeptide or nucleic acid) and a partner that is either a single component or a conjugate of 2 or more components (protein, carbohydrate, lipid DNA) as indicated below. Superantigen (Protein or DNA) Partner (Single Component or Conjugate) 1. DNA coding sequence 2. Polypeptide 3. Nucleic acid 4. Tumor associated Peptide 5. Tumor Antigen-MHC protein 6. LPS 7. Lipoarabinomannan 8. Ganglioside 9. Glycosphingolipid 10. Ganglioside-CD1 receptor 11. Glycosphingolipid-CD1 receptor 12. Glycosylceramide (e.g., Gal-Cer) 13. GalCer-CD1 receptor 14. Gal 15. Arg-Gly-Asp or Asn-Gly-Arg 16. iNOS 17. Gb2 or **Gb3** or Gb4 18. (Gb2 or **Gb3** or Gb4)-CD1 receptor 19. -GPI-(Gb2 or **Gb3** or Gb4) 20. -GPI-(Gb2 or **Gb3** or Gb4)-CD1 receptor_.sub.-- 21. Verotoxin 22. Verotoxin A or B Subunit.sub.-- 23. IFNa receptor peptide homologous to VT 24. CD19 peptide homologous to VT 25. LDL, VLDL, HDL, IDL 26. Apolipoproteins (e.g., Lp(a), apoB-100, apoB-48, apoE) OxyLDL, oxyLDL mimics, (e.g., 7.beta.-hydroperoxycholesterol, 7.beta.-hydroxycholesterol, 7-ketocholesterol, 5.alpha.-6.alpha.- epoxycholesterol, 7.beta.-hydroperoxy-choles-5-en-3.beta.-ol, 4- hydroxynonenal (4-HNE), 9-HODE, 13-HODE and cholesterol-9-HODE) 28. OxyLDL by products (e.g. lysolecithin, lysophosphatidylcholine, malondialdehyde, 4- hydroxynonenal) 29. LDL & oxyLDL receptors (e.g., LDL oxyLDL, acetyl-LDL, VLDL, LRP, CD36, SREC, LOX-1, macrophage scavenger receptors) 30. phytosphingosine, -GPI-phytosphinosine 31. tumor associated lipid antigens 32. glycolipid, proteolipid, glycosphingolipid, sphingolipid with inositolphosphate-containing head

groups, phytoglycolipids, mycoglycolipids, -GPI-sphinosines or GPI-lipids 33. sphingolipids with inositolphosphate-containing head groups having the general structure: ceramide-P-myoinositol-X with X referring to polar substituents comprising ceramide-p-inositol-mannose, inositol-1-P-(6)mannose(.- alpha. 1,2inositol-1P-(1)ceramide, (inositol-P)2-ceramide, inositol-P-inositol-P-ceramide, inositol-P-inositol-P-ceramide. 34. tumor associated glycan antigens consisting of peptidoglycans or glycan phosphotidyinositol (GPI) structures C. STCT or SAg-tumor peptide conjugates are incubated with in vivo immunized T cells or NKT cells for 2-4 days and then with IL-2 for 2-5 days. D. The tumor specific effector cells are then harvested and injected in doses of 10.sup.10-10.sup.12 every 3-7 days for 1-6 treatments. E. Viruses are transfected into tumor cells, accessory cells, antigen presenting cells, allogeneic or xenogeneic cells. They are pre-programmed with DNA for SAgs alone or in combination with genes given in D. They may also utilize the host genome to produce a new gene product as for example the host **-galactosyltransferase.** Viruses may include the following: 1. Adenoviruses. 2. Vaccinia virus. 3. Equine encephalitis virus. 4. Influenza virus. F. In an additional method, tumor associated antigens are bound to MHC class I positive cells and used to activate T cells. SAg-lipopolysaccharide complexes and SAg-glycosylceramide complexes are bound to CD1 or class II receptors on APCs. In addition, SAg-lipopolysaccharide complexes or SAg- glycosylceramide complexes are presented bound to class II positive APCs. Alternatively, unbound tumor associated antigen/SAg conjugates or fusion products are added at a 0.1 to 200 mg/ml dose for 2 days. This is followed by STCT incubation or by native or mutant SAg treatment for 2 days.

Detail Description Table CWU - DETL (12):

12 Superantigen (Protein or DNA) Partner (Single Component or Conjugate) 4. DNA coding sequence 5. Polypeptide 6. Nucleic acid 7. Tumor associated Peptide 8. Tumor Antigen-MHC protein 9. LPS 10. Lipoarabinomannan 11. Ganglioside 12. Glycosphingolipid 13. Ganglioside-CD1 receptor 14. Glycosphingolipid-CD1 receptor 15. Glycosylceramide (e.g., Gal-Cer) 16. GalCer-CD1 receptor 17. Gal 18. Arg-Gly-Asp or Asn-Gly-Arg 19 iNOS 20. Gb2 or **Gb3** or Gb4 21. (Gb2 or **Gb3** or Gb4)-CD1 receptor 22. -GPI-(Gb2 or **Gb3** or Gb4) 23. -GPI-(Gb2 or **Gb3** or Gb4)-CD1 receptor 24. Verotoxin 25. Verotoxin A or B Subunit.sub.-- 26. IFN.alpha. receptor peptide homologous to VT 27. CD19 peptide homologous to VT 28. LDL, VLDL, HDL, IDL 29. Apolipoproteins (e.g., Lp(a), apoB-100, apoB-48, apoE) 30. OxyLDL, oxyLDL mimics, (e.g., 7.beta.-hydroperoxycholesterol, 7.beta.-hydroxycholesterol, 7-ketocholesterol, 5.alpha.-6.alpha.- epoxycholesterol, 7.beta.-hydroperoxy-choles-5-en-3.beta.-ol, 4- hydroxynonenal (4-HNE), 9-HODE, 13-HODE and cholesterol-9-HODE) 31. OxyLDL by products (e.g. lysolecithin, lysophosphatidylcholine, malondialdehyde, 4- hydroxynonenal) 32. LDL & oxyLDL receptors (e.g., LDL oxyLDL, acetyl-LDL, VLDL, LRP, CD36, SREC, LOX-1, macrophage scavenger receptors) 33. phytosphingosine, -GPI-phytosphingosine 34. tumor associated lipid antigens 35. glycolipid, proteolipid, glycosphingolipid, sphingolipid with inositolphosphate -containing head groups, phytoglycolipids, mycoglycolipids, -GPI-sphingosines, -GPI-lipids 36. sphingolipids with inositolphosphate-containing head groups having the general structure: ceramide-P-myoinositol-X with X referring to polar substituents comprising ceramide-p-inositol-mannose,

inositol-1-P-(6)mannose(a- 1,2 inositol-1P-(1)ceramide,
(inositol-P)2-ceramide, inositol-P-inositol-P-ceramide,
inositol-P-inositol-P-ceramide. 37. tumor associated glycan antigens
consisting of peptidoglycans or glycan phosphotidyinositol (GPI) structures

PGPUB-DOCUMENT-NUMBER: 20030157108

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030157108 A1

TITLE: Glycoprotein compositions

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Presta, Leonard G.	San Francisco	CA	US	

APPL-NO: 10/ 277370

DATE FILED: October 22, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60337642 20011025 US

non-provisional-of-provisional 60347694 20020109 US

US-CL-CURRENT: 424/145.1, 424/141.1 , 424/155.1

ABSTRACT:

The present invention concerns compositions comprising a glycoprotein having an Fc region, wherein about 80-100% of the glycoprotein in the composition comprises a mature core carbohydrate structure which lacks fucose, attached to the Fc region of the glycoprotein. The preferred glycoprotein is an antibody or immunoadhesin.

---------- KWIC ---------

Detail Description Paragraph - DETX (18):
[0096] A "galactotransferase" is an enzyme that adds one or more galactose residue(s) to a glycoprotein. .beta.1,4-**galactosyltransferase** can add galactose residue(s) to the mature core carbohydrate structure.

Detail Description Paragraph - DETX (74):
[0152] A "B cell surface marker" herein is an antigen expressed on the surface of a B cell which can be targeted with an antibody which binds thereto. Exemplary B cell surface markers include the CD10, CD19, CD20, CD21, CD22, CD23, CD24, CD40, CD37, CD53, CD72, CD73, CD74, CDw75, CDw76, **CD77**, CDw78, CD79a, CD79b, CD80, CD81, CD82, CD83, CDw84, CD85 and CD86 leukocyte surface markers. The B cell surface marker of particular interest is preferentially

expressed on B cells compared to other non-B cell tissues of a mammal and may be expressed on both precursor B cells and mature B cells. In one embodiment, the marker is one, like CD20 or CD19, which is found on B cells throughout differentiation of the lineage from the stem cell stage up to a point just prior to terminal differentiation into plasma cells. The preferred B cell surface markers herein are CD19, CD20, CD22 and CD40.

PGPUB-DOCUMENT-NUMBER: 20030138807

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030138807 A1

TITLE: UDP-galactose: beta-D-galactose-R 4-alpha-D-galac-tosyltransferase, alpha4Gal-T1

PUBLICATION-DATE: July 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Clausen, Henrik	Holte		DK	
Steffensen, Rudi	Pandrup		DK	
Bennett, Eric Paul	Lyngby		DK	

APPL-NO: 10/ 217335

DATE FILED: August 8, 2002

RELATED-US-APPL-DATA:

child 10217335 A1 20020808

parent continuation-of PCT/DK01/00087 20010209 US UNKNOWN

non-provisional-of-provisional 60182037 20000211 US

US-CL-CURRENT: 435/6, 435/193 , 435/320.1 , 435/325 , 435/69.1 , 435/7.1 , 530/388.26 , 536/23.2

ABSTRACT:

A novel gene defining a novel enzyme UDP-Galactose: .beta.-D-Galactose-R 4-.alpha.-D-**galactosyltransferase,** termed .alpha.4Gal-T1, with unique enzymatic properties is disclosed. The invention discloses isolated DNA molecules and DNA constructs encoding .alpha.4Gal-T1 and derivatives thereof by way of amino acid deletion, substitution or insertion exhibiting .alpha.4Gal-T1 activity, as well as cloning and expression vectors including such DNA, cells transfected with vectors, and recombinant methods for providing .alpha.4Gal-T1. The enzyme .alpha.4Gal-T1 and .alpha.4Gal-active derivatives thereof are disclosed. Further, the invention discloses methods of obtaining .alpha.1, 4galactosyl glycosylated glycosphingolipids by use of an enzymatically active .alpha.4Gal-T1 protein thereof or by using cells stably transfected with a vector including DNA encoding an enzymatically active .alpha.4Gal-T1 protein as an experession system for recombinant production of such glycosphingolipids. Also a method for the identification of DNA sequence variations in the .alpha.4Gal-T1-coding exon by PCR, and detecting the presence of DNA sequence variation, are disclosed.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

A novel gene defining a novel enzyme UDP-Galactose: .beta.-D-Galactose-R 4-.alpha.-D-**galactosyltransferase,** termed .alpha.4Gal-T1, with unique enzymatic properties is disclosed. The invention discloses isolated DNA molecules and DNA constructs encoding .alpha.4Gal-T1 and derivatives thereof by way of amino acid deletion, substitution or insertion exhibiting .alpha.4Gal-T1 activity, as well as cloning and expression vectors including such DNA, cells transfected with vectors, and recombinant methods for providing .alpha.4Gal-T1. The enzyme .alpha.4Gal-T1 and .alpha.4Gal-active derivatives thereof are disclosed. Further, the invention discloses methods of obtaining .alpha.1, 4galactosyl glycosylated glycosphingolipids by use of an enzymatically active .alpha.4Gal-T1 protein thereof or by using cells stably transfected with a vector including DNA encoding an enzymatically active .alpha.4Gal-T1 protein as an experession system for recombinant production of such glycosphingolipids. Also a method for the identification of DNA sequence variations in the .alpha.4Gal-T1-coding exon by PCR, and detecting the presence of DNA sequence variation, are disclosed.

Summary of Invention Paragraph - BSTX (2):

[0001] The present invention relates generally to the biosynthesis of glycans found as free oligosaccharides or covalently bound to proteins and glycosphingolipids. This invention is more particularly related to nucleic acids encoding an UDP-D-galactose: .beta.-D-galactose-R 4-.alpha.-D-**galactosyltransferase** (.alpha.4Gal-transferase), which add galactose to the hydroxy group at carbon 4 of D-galactose (Gal). This invention is more particularly related to a gene encoding the blood group P.sup.k (**Gb3**) synthase, termed .alpha.4Gal-T1, probes to the DNA encoding .alpha.4Gal-T1, DNA constructs comprising DNA encoding .alpha.4Gal-T1, recombinant plasmids and recombinant methods for producing .alpha.4Gal-T1, recombinant methods for stably transfecting cells for expression of .alpha.4Gal-T1, and methods for identification of DNA polymorphism in patients.

Summary of Invention Paragraph - BSTX (5):

[0003] In contrast, the P, P.sup.k, and p antigens constitute intermediate steps in biosynthesis of globo-series glycollpids and give rise to P.sub.1.sup.k, P.sub.2.sup.k, and p phenotypes (Naiki and Marcus, 1974). While the rare .sup.Pk phenotype show the same frequency of P1 antigen expression as individuals expressing the P antigen, the p phenotype is always associated with lack of P.sub.1 antigen expression. Extensive studies of the chemistry, biosynthesis, and genetics of the P blood group system identified the antigens as being exclusively found on glycolipids, with the blood group specificity being synthesized by at least two distinct glycosyltransferase activities; UDP-galactose: .beta.-D-galactosyl-.beta.1-R 4-.alpha.-D-**galactosyltransferase** (.alpha.4Gal-T) activity(ies) for Pk and P1 syntheses and UDP-GalNAc: **Gb3** 3-.beta.-N-acetylgalactosaminyltransferase activity (EC 2.4.1.79) for P synthesis [for reviews see (Issitt and Anstee, 1998; Bailly and Bouhors, 1995)]. At least two independent gene loci, P and P.sub.1P.sup.k, are involved

in defining these antigens. The P blood group associated LKE antigen shown to be the extended sialylated Gal-globoside structure (Tippett et al., 1986), may involve polymorphism in an .alpha.2,3sialyltransferase activity.

Summary of Invention Paragraph - BSTX (8):

[0006] Access to the Pk .alpha.4Gal-transferase gene would allow production of efficient enzymes for use in galactosylation of glycosphingolipids, oligosaccharides, and glycoproteins. Such enzymes could be used, for example, in pharmaceutical or other commercial applications that require enzymatic galactosylation of these or other substrates in order to produce appropriately glycosylated glycoconjugates having particular enzymatic, immunogenic, or other biological and/or physical properties. The P blood group system is implicated in important biological phenomena. Blood group p individuals have strong anti-P.sub.1PP.sup.k IgG antibodies and these are implicated in high incidence of spontaneous abortions (Yoshida et al., 1994). The globoseries glycolipid antigens constitute major receptors for microbial pathogens with the Gal.alpha.1-4Gal linkage being an essential part of the receptor site (for a review see (Karlsson, 1998)). The P.sup.k glycolipid is the **CD77** antigen, a B cell differentiation antigen, which is able to transduce a signal leading to apoptosis of the cells (Mangeney et al., 1993). Furthermore, the association of this glycolipid with the type I interferon receptor or with the HIV-1 co-receptor, CXCR4, seems to be crucial for the functions of these receptors (Taga et al., 1997; Puri et al., 1999). Cloning of the P.sup.k synthase is an important step toward understanding the biological roles of the globo-series class of glycolipids, and a first step in elucidating the molecular genetics of the P blood group system. Availability of the P.sup.k synthase gene is important for elucidating the many biological roles of the globo-series class of glycolipids, and may offer new avenues for diagnostic and therapeutic measures.

Summary of Invention Paragraph - BSTX (9):

[0007] Consequently, there exists a need in the art for UDP-galactose: .beta.-D-galactose-R 4-.alpha.-D-**galactosyltransferase** and the primary structure of the gene encoding this enzyme. The present invention meets this need, and further presents other related advantages, as described in detail below.

Summary of Invention Paragraph - BSTX (11):

[0008] The present invention provides isolated nucleic acids encoding human UDP-galactose: .beta.-D-galactose-R 4-.alpha.-D-**galactosyltransfera- se** (.alpha.4Gal-T1), including cDNA and genomic DNA. .alpha.4Gal-T1 represents the first cloned and expressed eukaryote .alpha.4Gal-T gene. The complete nucleotide sequence of .alpha.Gal-T1, is set forth in SEQ ID NO:10 and FIG. 1.

Summary of Invention - Table CWU - BSTL (1):

1TABLE I Structures of glycosphingolipids referred to in this study.sup.a P blood group Structure antigen CDH, LacCer Gal.beta.1-4Glc.beta.-1Cer p CTH, Gb.sub.3 Gal.alpha.1-4Ga.beta.1-4Glc.beta.1-1Cer pk Globoside GalNAc.beta.1-3Gab.alpha.1-4Gal.beta.1-4Glc.beta.1-1Cer P Sialyl-Gal-Globoside

NeuAc.alpha.2-3Gal.beta.1-3GalNAc.beta.1-3Gal.alpha.-
1-4Gal.beta.1-4Glc.beta.1-1Cer LKE Paragloboside, PG
Gal.beta.1-4GlcNAc.beta.1-3Gal.beta.1-4Glc.beta.1-1Cer P.sub.1
Gal.alpha.1-4Gal.beta.1-4GlcNA.beta.1-3Gal.beta.1-4Glc.beta.1-1Cer P.sub.1
.sup.aKey: CDH, ceramide dihexoside (lactosylceramide, LacCer); CTH, Ceramide trihexoside (Gb.sub.3, **globotriaosylceramide**); globoside, Gb4 (globotetraosylcerarnide); Cer, ceramide; Gal, D-galactose; Glc, D-glucose; GalNAc, N-acetyl-D-galactosamine; GlcNAc, N-acetyl-D-glucosamine; NeuAc, N-acetylneuraxninic acid.

Brief Description of Drawings Paragraph - DRTX (8):

[0025] FIG. 7 illustrates cell surface expression of P.sup.k/**CD77** antigen in Namalwa cells after transient transfection of .alpha.4Gal-T1. Constructs p#5, #45, and #67, as well as empty pDR2 vector were electroporated in Namalwa cells and expression of P.sup.k/**CD77** antigen was tested after 48 hours. Cells were labeled with 1A4 monoclonal antibody and GAM-FITC (grey histograms) or with GAM-FITC alone (empty histograms) and analysed with a FACSCalibur flow cytometer.

Detail Description Paragraph - DETX (11):

[0035] 8. A "donor substrate" is a molecule recognized by, e.g., a .alpha.1,4galactosyltransferase and that contributes a galactose moiety for the transferase reaction. For .alpha.4Gal-T1, a donor substrate is UDP-galactose. An "acceptor substrate" is a molecule, preferably a saccharide or oligosaccharide, that is recognized by, e.g., a **galactosyltransferase** and that is the target for the modification catalyzed by the transferase, i.e., receives the galactose moiety. For .alpha.4Gal-T1, acceptor substrates include without limitation glycosphingolipids, oligosaccharides, glycoproteins, glycopeptides, and comprising the sequences Gal.beta.1-4Glc, or Gal.beta.1-3Glc.

Detail Description Paragraph - DETX (23):

[0047] The presented data, however, do not explain the molecular genetic basis of the P.sub.1 blood group polymorphism. Although the P.sub.1 polymorphism is linked to the same chromosomal localization as .alpha.4Gal-T1, we found no genetic polymorphisms in the .alpha.4Gal-T1 gene associated with the P.sub.1+/- phenotypes, and recombinant .alpha.4Gal-T1 variants did not express P.sub.1 synthase activity in vitro (Tables II and III, FIG. 4). Searching the available chromosome 22 sequence did not reveal additional homologous genes. Thus, essentially two possibilities exist: i) .alpha.4Gal-T1 can be activated by another non-homologous polymorphic gene or gene product and function as a P.sub.1 synthase; or ii) a second polymorphic .alpha.4Gal-T gene, which is non-homologous to .alpha.4Gal-T1, exists. The former possibility has a precedent in two members of the .beta.4Gal-T gene family, .beta.4Gal-T1 and -T2, both of which are modulated by .alpha.-lactalbumin to change their, function from N-acetyllactosamine synthases to lactose synthases (Brodbeck et al., 1967; Brew et al., 1968; Almeida et al., 1997). Binding of .alpha.-Lactalbumin to these **galactosyltransferases** changes the acceptor substrate specificity from GlcNAc to Glc, but also to some degree affects the donor substrate specificity to include UDP-GalNAc (Do et al., 1995). The induction of .beta.4Gal-T1 by a-lactalbumin to enable it to function as a

lactose synthase is combined with a complex regulatory mechanism by which the .beta.4Gal-T1 synthase is 100-fold upregulated in mammary glands (Charron et al., 1998). As lactose is the major nutrient in milk, this complex model for its synthesis appears to be in accordance with the biological function. The P.sub.1 antigen has only been detected as a minor glycosphingolipid component, and no biological function for this polymorphic antigen has been identified. It therefore at present may seem less likely that a unique modulator of the .alpha.4Gal-T1 gene has evolved. The second possibility of the existence of another polymorphic non-homologous .alpha.4Gal-T gene located in the same chromosomal region implies that the encoded .alpha.4Gal-T functions as both P.sup.k and P.sub.1 synthases. This is based on the findings that p individuals do not produce P1 antigens, and it is supported by the finding that erythrocytes of P.sub.1 individuals contain relative less LacCer and more **Gb3** than P.sub.2 individuals (Fletcher et al., 1979). Generally, glycosyltransferases with similar functions are encoded by homologous glycosyltransferase gene families (Amado et al., 1999), however, recently two non-homologous .beta.3GlcNAc-transferases both functioning as poly-N-acetyllactosamine synthases have been identified (Sasaki et al., 1997; Zhou et al., 1999).

Detail Description Paragraph - DETX (27):

[0051] Expression of full coding constructs of .alpha.4Gal-T1.sup.37M and .alpha.4Gal-T1.sup.37V in insect cells resulted in marked increase in **galactosyltransferase** activity with CDH, compared to uninfected cells or cells infected with a control construct (FIG. 4). In contrast, no activity was found with the .alpha.4Gal-T1.sup.183K gene from p individuals. Importantly, neither .alpha.4Gal-T1.sup.37M or .alpha.4Gal-T1.sup.37V constructs conferred .alpha.4Gal-T activity with the neolacto-series (paragloboside) glycolipid acceptor for P.sub.1 synthase activity (FIG. 4). The assay conditions for measuring P.sup.k and P.sub.1 synthase activity was the same except substitution of the acceptor substrate, and these conditions were previously used to demonstrate both activities in kidney extracts from P.sub.1+ and P.sub.1- individuals (Bailly et al., 1992). The soluble, secreted construct encoding residues 47-353 did not result in active .alpha.4Gal-T activity (data not shown). Attempts to obtain complete conversion of CDH to CTH were unsuccessful, but a 1-D .sup.1H-NMR spectrum of the purified reaction mixture (not shown) clearly exhibited H-1 resonances diagnostic for CTH at levels approximately 30% of those of the CDH acceptor substrate. Thus, in addition to major resonances at 4.205 ppm (.sup.3J.sub.1,2=7.2 Hz) and 4.165 ppm (.sup.3J.sub.1,2=7.9 Hz), corresponding to H-1 of Gal.beta.4 and Glc.beta.1of CDH, minor resonances were observed at 4.794 ppm (.sup.3J.sub.1,2=3.7 Hz) and 4.258 ppm (.sup.3J.sub.1,2=6.9 Hz), corresponding to H-1 of Gal.alpha.4 and Gal.beta.4 of CTH (the chemical shift of Glc.beta.1 H-1 is not affected by the addition of the terminal Gal.alpha.4 residue). The chemical shift and .sup.3J.sub.1,2 coupling of the downfield H-1 resonance are particularly characteristic for Gal.alpha.4 of CTH and other globo-series glycosphingolipids (Dabrowski et al., 1980; Kannagi et al., 1983). Analysis with a number of saccharide acceptors including lactose, lactosamine, and benzyl .beta.-lacto-side, revealed no significant activity over background values.

Detail Description Paragraph - DETX (29):

[0053] Northern analysis with mRNA from 12 human organs revealed a ubiquitous expression pattern with high expression in kidney and heart and low expression in other organs (FIG. 5). The kidney primarily synthesizes globoseries glycosphingolipids (Clausen and Hakomori, 1989). Analysis of 8 human cell lines revealed an expression pattern correlating with .alpha.4Gal-T1 activity and cell surface expression of P.sup.k antigen (FIG. 6) (Taga et al., 1995b; Taga et al., 1995a). Ramos cells have the highest antigen expression and .alpha.4Gal-T activity, and strong expression of .alpha.4Gal-T1. In contrast, Namalwa cells that do not produce P.sup.k antigens and have no measurable .alpha.4Gal-T activity, showed no expression of .alpha.4Gal-T1. However, transient transfection of Namalwa cells with the full coding constructs of .alpha.4Gal-T1 (#67 and #45) clearly resulted in P.sup.k/**CD77** expression as revealed by FACS analysis (FIG. 7).

Detail Description Paragraph - DETX (143):

[0162] The three full coding constructs #67, #45, and p#5, were cloned into pDR2 (Clontech, USA). Insert was excised from pBKs with BamHI/XhoI and inserted into the BamHI/SalI sites of pDR2. Transient transfection of 5.times.10.sup.6 Namalwa cells with 20 .mu.g cDNA was done by double-pulse electroporation using an Easy-cell ject+ (Eurogentec, France). Expression of **CD77**/P.sup.k antigen was evaluated by FACS analysis on a FACSCalibur (Beckton-Dickinson, USA) using 1A4 monoclonal antibody (Wiels, 1997).

Detail Description Paragraph - DETX (151):

[0167] Almeida, R., Amado, M., David, L., et al. A Family of Human .beta.4-**Galactosyltransferases**: Cloning and expression of two novel UDP-Galactose: .beta.-N-Acetylglucosamine .beta.1,4-**Galactosyltransferase- s**, .beta.4Gal-T2 and .beta.4Gal-T3. J.Biol.Chem. 272:31979-31992, 1997.

Detail Description Paragraph - DETX (152):

[0168] Amado, M., Almeida, R., Schwientek, T. and Clausen, H. Identification and Characterization of Large **Galactosyltransferase** Gene Families: **Galactosyltransferases** for all functions. Biochim Biophys Acta in press:1999.

Detail Description Paragraph - DETX (160):

[0176] Charron, M., Shaper, J. H. and Shaper, N. L. The increased level of betal,4-**galactosyltransferase** required for lactose biosynthesis is achieved in part by translational control. Proc Natl Acad Sci U.S.A 95:14805-14810, 1998.

Detail Description Paragraph - DETX (164):

[0180] Do, K. Y., Do, S. I. and Cummings, R. D. Alpha-lactalbumin induces bovine milk beta 1,4-**galactosyltransferase** to utilize UDP-GalNAc. J.Biol.Chem. 270:18447-18451, 1995.

Detail Description Paragraph - DETX (167):

[0183] Iizuka, S., Chen, S. H. and Yoshida, A. Studies on the human blood group P system: an existence of UDP-Gal:lactosylceramide alpha 1----4

galactosyltransferase in the small p type cells. Biochea Biophys Res Commun. 137:1187-1195, 1986.

Detail Description Paragraph - DETX (175):
[0191] Lopez, M., Gazon, M., Juliant, S., et al. Characterization of a UDP-Gal:Galbeta1-3GalNAc alphal, 4-**galactosyltransferase** activity in a Mamestra brassicae cell line. J Biol Chem. 273:33644-33651, 1998.

Detail Description Paragraph - DETX (177):
[0193] Mangeney, M., Lingwood, C. A., Taga, S., Caillou, B., Tursz, T. and Wiels, J. Apoptosis induced in Burkitt's lymphoma cells via **Gb3/CD77**, a glycolipid antigen. Cancer Res 53:5314-5319, 1993.

Detail Description Paragraph - DETX (188):
[0204] Puri, A., Hug, P., Jernigan, K., Rose, P. and Blumenthal, R. Role of glycosphingolipids in HIV-1 entry: requirement of globotriosylceramide (**Gb3**) in CD4/CXCR4-dependent fusion. Biosci.Rep. 19:317-325, 1999.

Detail Description Paragraph - DETX (190):
[0206] Taga, S., Carlier, K., Mishal, Z., et al. Intracellular signaling events in **CD77**-mediated apoptosis of Burkitt's lymphoma cells. Blood 90:2757-2767, 1997.

Detail Description Paragraph - DETX (194):
[0210] Wakarchuk, W. W., Cunningham, A., Watson, D. C. and Young, N. M. Role of paired basic residues in the expression of active recombinant **galactosyltransferases** from the bacterial pathogen Neisseria meningitidis. Protein Eng. 11:295-302, 1998.

Detail Description Paragraph - DETX (196):
[0212] Wiels, J. **CD77** Final Workshop. In: Leukocyte Typing VI, edited by Kishimoto, T. London: Garland Publishing Inc., 1997, p. 175-177.

Detail Description Paragraph - DETX (201):
[0217] Zhou, D., Dinter, A., Gutierrez, G. R., et al. A beta-1,3-N-acetylglucosaminyltransferase with poly-N-acetyllactosamine synthase activity is structurally related to beta-1,3-**galactosyltransfera- ses**. Proc Natl Acad Sci U.S.A 96:406-411, 1999.

Claims Text - CLTX (1):
1. An isolated nucleic acid encoding UDP-galactose: .beta.-D-Galactose .alpha.1-4-D-**galactosyltransferase** (.alpha.4Gal-T1) or a fragment hereof preserving its function as .alpha.4Gal-T1.

PGPUB-DOCUMENT-NUMBER: 20030119052

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030119052 A1

TITLE: METHODS FOR IDENTIFYING A DESIRED ENZYMATIC ACTIVITY

PUBLICATION-DATE: June 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
SHORT, JAY M.	ENCINITAS	CA	US	

APPL-NO: 09/ 467740

DATE FILED: December 20, 1999

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

child 09467740 A1 19991220

parent continuation-of 08503606 19950718 US GRANTED

parent-patent 6004788 US

US-CL-CURRENT: 435/7.1, 435/18 , 435/183 , 435/25

ABSTRACT:

Recombinant enzyme libraries and kits where a plurality of enzymes are each characterized by different physical and/or chemical characteristics and classified by common characteristics. The characteristics are determined by screening of recombinant enzymes expressed by a DNA library produced from various microorganisms.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (139):
[0139] d. Glycoside synthesis using UDP-**galactosyl transferase**

Detail Description Table CWU - DETL (5):
5TABLE 4 25 4-methyl umbelliferone wherein R = G2 .beta.-D-galactose .beta.-D-glucose .beta.-D-glucuronide **GB3** .beta.-D-cellotrioside .beta.-B-cellobiopyranoside GC3 .beta.-D-galactose .alpha.-D-galactose GD3

.beta.-D-glucose .alpha.-D-glucose GE3 .beta.-D-glucuronide GI3 .beta.-D-N,N-diacetylchitobiose GJ3 .beta.-D-fucose .alpha.-L-fucose .beta.-L-fucose GK3 .beta.-D-mannose .alpha.-D-mannose non-Umbelliferyl substrates GA3 amylose [polyglucan .alpha.1,4 linkages], amylopectin [polyglucan branching .alpha.1,6 linkages] GF3 xylan [poly 1,4-D-xylan] GG3 amylopectin, pullulan GH3 sucrose, fructofuranoside

PGPUB-DOCUMENT-NUMBER: 20030068323

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030068323 A1

TITLE: Hybrid compositions for intracellular targeting

PUBLICATION-DATE: April 10, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Arab, Sara	North York		CA	
Lingwood, Clifford A.	Toronto		CA	
Khine, Aye-Aye	Concord		CA	

APPL-NO: 10/ 298408

DATE FILED: November 18, 2002

RELATED-US-APPL-DATA:

child 10298408 A1 20021118

parent continuation-of 08975953 19971121 US GRANTED

parent-patent 6482586 US

non-provisional-of-provisional 60031668 19961122 US

non-provisional-of-provisional 60061050 19971003 US

non-provisional-of-provisional 60061044 19971004 US

US-CL-CURRENT: 424/178.1, 514/44 , 514/8

ABSTRACT:

Hybrid compounds comprising a first domain and a second domain are provided. The first domain and the second domain are preferably covalently linked, and the first domain comprises a domain which is capable of specific binding to Gb.sub.3; and the second domain comprising a moiety selected from the group consisting of drug moiety, a nucleic acid, a probe, a polypeptide, and a hook, with the proviso that the second domain is not a verotoxin or a fragment thereof. Mehtods of preapring and using the hybrid compounds are also provided.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. 119(e) to co-pending U.S. provisional application Serial No. 60/031,668, filed Nov. 22, 1996; No.

60/061,050, filed Oct. 3, 1997; and No. 60/061,044, filed Oct. 4, 1997; the contents of all of which are hereby incorporated by reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (4):

[0004] The E coli derived family of verotoxins (or Shiga-like toxins) comprise VT1, VT2 and VT2c, which are involved in the etiology of microvascular disease in man (8), primarily in the very young and elderly (9), and VT2e which causes edema disease in pigs (10). The glycolipid **globotriaosylceramide** (gala1-4galb1-4glc cer.-Gb.sub.3) at the plasma membrane is the specific receptor for all verotoxins and mediates the internalization of verotoxin (VT1) into susceptible cells by capping and receptor-mediated endocytosis (RME) (11). Verotoxin is the only glycolipid binding ligand that is internalized into eukaryotic cells by means of RME (12-14). In addition to receptor concentration, both heterogeneous fatty acid composition of Gb.sub.3 (15, 16) and phospholipid chain length within the phospholipid bilayer (17) play important roles in binding and internalization of VT. Molecular modeling studies of the Gb.sub.3 binding site in the B subunit (18) show that different conformers of membrane Gb.sub.3 may bind in different sites. Such conformers may be related to the Gb.sub.3 fatty acid content and membrane phospholipid microenvironment (18-20).

Detail Description Paragraph - DETX (3):

[0015] In one aspect, the invention provides a hybrid compounds. The hybrid compound includes a first domain and a second domain; the first and second domains are, preferably, covalently linked. The first domain is a binding domain capable of specific binding to **globotriaosylceramide** (Gb.sub.3) and being internalized into a cell which expresses Gb.sub.3 on the cell surface. The second domain is a functional domain which includes a molecular moiety which is to be delivered into the cell, e.g., to the cell nucleus. The second domain is preferably not a verotoxin, a verotoxin subunit, or a fragment thereof. The second domain can be, for example, a drug moiety (e.g., a drug molecule bound to the first domain), a nucleic acid (e.g., a gene which encodes an exogenous protein, or a nucleic acid which regulates gene expression in a cell, such as antisense nucleic acid, repressors, or trans activators), a probe (such as a fluorescent probe), a protein, and the like. The second domain can also be a domain which functions as a handle or hook for complexation or binding to another moiety or moieties. For example, the second domain can be a member of a specific binding pair (such as biotin/streptavidin, hormone/receptor, binding protein/ligand, and the like), which can be complexed with or bound to the other member of the specific binding pair, which can, in turn, be bound to a moiety which is desired to be delivered into the cell.

Detail Description Paragraph - DETX (7):

[0019] As described in more detail hereinbelow, the invention further provides methods for altering the intracellular target of a hybrid compound of the invention. Thus, for example, treatment of a cell with sodium butyrate can alter the intracellular destination of VT-B, and therefore a hybrid compound of

the invention. As described infra, in certain cells, VT-B is normally transported to components of the Golgi apparatus in the absence of sodium butyrate; however, in the presence of butyrate, VT-B is transported to elements of the endoplasmic reticulum and/or the nuclear envelope. As is also described in more detail hereinbelow, this effect is believed to be due, at least in part, to alterations in the fatty acid composition of the cell surface glycolipid Gb.sub.3. The invention contemplates the selective transport of a hybrid compound of the invention to a selected location in the cell, e.g., the nuclear membrane or nucleus. This feature of the invention is especially useful for gene therapy applications (or antisense treatments) in which the nuclear genome, rather than the DNA in the cytoplasm or cytoplasmic organelles, is to be targeted. Accordingly, in certain embodiments, the invention contemplates treatment of a cell with a compound (e.g., butyrate), or under conditions, capable of effecting a change in the fatty acid composition of **Gb3**, and thereby promoting selective transport of a hybrid compound of the invention to a pre-selected intracellular location (e.g., the nucleus).

Detail Description Paragraph - DETX (10):

[0022] The first domain of the hybrid compounds of the invention comprises a domain which is capable of specific binding to **globotriaosylceramide** (Gb.sub.3), and is capable of being internalized into a cell which expresses Gb.sub.3 on the cell surface; such a domain will for convenience sometimes be referred to herein as a "VT binding domain" although, as described herein, first domains suitable for use in the invention are not limited to verotoxins or fragments thereof. Domains suitable for use as a first domain of a hybrid compound of the invention include native verotoxins (VTs), subunits of verotoxins (e.g., VT-B subunit) which bind to Gb.sub.3, and polypeptides comprising amino acid sequences homologous to and/or derived from the amino acid sequence of a native VT binding domain, which can include more, fewer (e.g., a deletion or truncation), or an equal number (e.g., point mutations) of amino acids than a full length VT binding domain protein, while retaining substantial specific binding affinity for Gb.sub.3 (or Burkitt's lymphoma associated antigen (BLA) (Nudelman, et al. Science 220: 509 (1983), also known as the B-cell differentiation antigen **CD77**). Thus, a "VT binding domain", as used herein, refers to the Gb.sub.3 receptor binding subunit of verotoxins or homologous domains which have Gb.sub.3 binding activity. It will be appreciated that certain proteins or polypeptides are known which have substantial homology to verotoxin binding domains (e.g., CD19, a 95 kDa immunoglobulin superfamily integral membrane glycoprotein present on the cell surface of human B lymphocytes from the early stage of B-cell development to the terminal differentiation of B-cells to plasma cells (Nadler, et al. J. Immunol. 131: 244-250 (1983); Lingwood, C. A. (1996) Trends in Microbiol. 4(4):147-153; Maloney, M. D. and Lingwood, C. A. (1994) J. Exp. Med. 180:191-201; Nyholm, P. G., Magnusson, G. and Lingwood, C. (1996) Chem. Biol. 3:263-275) and can bind to Gb.sub.3 or Gb.sub.3-like cell surface moieties; use of such homologous proteins or polypeptides is contemplated in the hybrid compounds of the invention. In one embodiment, the first domain of a hybrid compound of the invention is at least about 30%, 40%, more preferably at least about 50%, 60%, even more preferably at least about 70%, 80%, yet even more preferably at least about 90%, and most preferably at least about 95% (or more) homologous to a Gb.sub.3 binding domain of a native verotoxin (or verotoxin subunit). Typically, biologically active portions comprise a domain or motif

with at least one activity of a VT binding domain. A biologically active portion of a VT binding domain protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

Detail Description Paragraph - DETX (95):

[0106] After trypsinization, cells (.about.1.times.10.sup.6) were washed with PBS three times, resuspended in a minimum volume, and extracted with 20 volumes of chloroform/methanol (C/M) 2:1 by vol. The extract was partitioned against water and the lower phase partitioned again against theoretical upper phase. The combined lower phase was then evaporated, saponified with 1 N NaOH in methanol and glycolipids reextracted as above. The dried lower phase was dissolved in CM 98:2 and separated by silica chromatography (32). The column was washed extensively with chloroform and glycolipid eluted in acetone/methanol (9:1 by vol.). **Gb3** present was detected by tlc overlay binding with VT binding domain1 (16).

Detail Description Paragraph - DETX (130):

[0137] The pattern of FITC-VT binding domain1 B localization was completely distinct in the SF-539 and XF-498 astrocytoma cell lines, despite comparable **Gb3** content and cell surface binding at 4.degree. C. In the more VT binding domain sensitive SF-539 cells, at 37.degree. C., intracellular FITC-VT binding domain1-B accumulated around the nucleus and apparently within the nucleus. However, the intracellular localization of FITC-VT binding domain1-B in XF-498 cells was in a juxtanuclear location, consistent with Golgi localization. Double labeling confocal microscopy verified the targeting of VT binding domain1B to the the nuclear envelope/ER and nucleus in SF-539 cells. In SF-539 cells, RITC-B also colocalized with anti BIP (GRP 78), a marker for the ER(41) as a ring around the nucleus. The punctate staining for VT binding domain1B and BIP for the most part was coincident, however some BIP staining showed no corresponding toxin localization and vice versa. The latter result is likely due to VT binding domain1B localization in part, in intermediate compartment vesicles (between Golgi and ER) (42). In addition, intranuclear staining is clearly seen for VT binding domain1B but not for BIP. Staining for ERGIC 53, a marker of the intermediate compartment vesicles in part, colocalized with VT binding domain1B staining. In contrast, no nuclear staining was seen for XF-498. Double labeling confocal microscopy showed that the juxtanuclear structure labeled in XF 498 cells was colocalized with Con A labeled Golgi. VT binding domain1B was restricted to the Golgi and did not localize with the additional Con A. staining of the ER around the nucleus.

Detail Description Paragraph - DETX (153):

[0153] 5. Mangeney, M., C. A. Lingwood, B. Caillou, S. Taga, T. Tursz and J. Wiels. 1993. Apoptosis induced in Burkitt's lymphoma cells via **Gb3/CD77,** a glycolipid antigen., Cancer Res, 53, 5314.

Detail Description Paragraph - DETX (203):

[0203] 55. Jacewicz, M. S., D. W. K. Acheson, M. Mobassaleh, A. Donohue-Rolfe, K. A. Balasubramanian and G. T. Keusch. 1995. Maturational regulation of **globotriaosylceramide,** the Shiga-like toxin 1 receptor, in

cultured human gut epithelial cells, J Clin Invest, 96, 1328.

Detail Description Paragraph - DETX (208):
[0208] 60. Maloney, M. D. and C. A. Lingwood. 1994. CD19 has a potential **CD77** (globotriaosyl ceramide)-binding site with sequence similarity to verotoxin B-subunits: Implications of molecular mimicry for B cell adhesion and enterohemorrhagic Escherichia coli pathogenesis, J Exp Med, 180, 191.

Detail Description Paragraph - DETX (209):
[0209] 61. Khine, A. A. and C. A. Lingwood. submitted. **CD77** Dependent Retrograde Transport of CD19 to the Nuclear Membrane: Functional Relationship between **CD77** and CD19 during Germinal Center B-cell Apoptosis.,

Detail Description Paragraph - DETX (215):
[0215] 67. Burger, K., P. van der Bijl and G. van Meer. 1996. Topology of sphingolipid **galactosyl transferase** in ER and Golgi:transbilayer movement of monohexyl sphingolipids is required for higher glycosphingolipid biosynthesis., J Cell Biol, 133, 15.

US-PAT-NO: 6566050

DOCUMENT-IDENTIFIER: US 6566050 B2

TITLE: Enzyme kits and libraries

DATE-ISSUED: May 20, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Short; Jay M.	Rancho Santa Fe	CA	N/A	N/A

APPL-NO: 09/ 861267

DATE FILED: May 18, 2001

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a divisional of U.S. patent application Ser. No. 09/467,740, filed Dec. 20, 1999, which is a continuation of U.S. patent application Ser. No. 08/503,606, filed Jul. 18, 1995, issued as U.S. Pat. No. 6,004,788, the disclosures of which are incorporated herein by reference in their entirety.

US-CL-CURRENT: 435/4, 435/6

ABSTRACT:

Recombinant enzyme libraries and kits where a plurality of enzymes are each characterized by different physical and/or chemical characteristics and classified by common characteristics. The characteristics are determined by screening of recombinant enzymes expressed by a DNA library produced from various microorganisms.

5 Claims, 4 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

---------- KWIC ---------

Brief Summary Text - BSTX (55):

3 Glycosidase/Glycosyl Transferase a. Sugar/polymer synthesis b. Cleavage of glycosidic linkages to form mono, di- and oligosaccharides c. Synthesis of complex oligosaccharides d. Glycoside synthesis using UDP-**galactosyl**

transferase e. Transglycosylation of disaccharides, glycosyl fluorides, aryl galactosides f. Glycosyl transfer in oligosaccharide synthesis g. Diastereoselective cleavage of .beta.-glucosylsulfoxides h. Asymmetric glycosylations i. Food processing j. Paper processing

Detailed Description Paragraph Table - DETL (5):

TABLE 4 ##STR28## 4-methyl umbelliferone wherein R = G2 .beta.-D-galactose .beta.-D-glucose .beta.-D-glucuronide **GB3** .beta.-D-cellotrioside .beta.-B-cellobiopyranoside GC3 .beta.-D-galactose .alpha.-D-galactose GD3 .beta.-D-glucose .alpha.-D-glucose GE3 .beta.-D-glucuronide GI3 .beta.-D-N,N-diacetylchitobiose GJ3 .beta.-D-fucose .alpha.-L-fucose .beta.-L-fucose GK3 .beta.-D-mannose .alpha.-D-mannose non-Umbelliferyl substrates GA3 amylose [polyglucan .alpha.1,4 linkages], amylopectin [polyglucan branching .alpha.1,6 linkages] GF3 xylan [poly 1,4-D-xylan] GG3 amylopectin, pullulan GH3 sucrose, fructofuranoside

US-PAT-NO: 6528249

DOCUMENT-IDENTIFIER: US 6528249 B1

TITLE: Protein activity screening of clones having DNA from uncultivated microorganisms

DATE-ISSUED: March 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Short; Jay M.	Encinitas	CA	N/A	N/A

APPL-NO: 09/ 713176

DATE FILED: November 14, 2000

PARENT-CASE:

This application is a continuation of application Ser. No. 08/988,224, filed Dec. 10, 1997, now U.S. Pat. No. 6,280,926, which is a divisional application of U.S. patent application Ser. No. 08/657,409, filed on Jun. 3, 1996, now U.S. Pat. No. 5,958,672, which was a continuation-in-part of U.S. application Ser. No. 08/568,994, filed on Dec. 7, 1995, now abandoned, continuation-in-part of U.S. application Ser. No. 08/503,606, filed on Jul. 18, 1995, now U.S. Pat. No. 6,004,788.

US-CL-CURRENT: 435/4, 435/6

ABSTRACT:

Disclosed is a process of screening clones having DNA from an uncultivated microorganism for a specified protein, e.g. enzyme, activity by screening for a specified protein, e.g. enzyme, activity in a library of clones prepared by (i) recovering DNA from a DNA population derived from at least one uncultivated microorganism; and (ii) transforming a host with recovered DNA to produce a library of clones which is screened for the specified protein, e.g. enzyme, activity.

25 Claims, 5 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 5

---------- KWIC ---------

Detailed Description Text - DETX (40):

3. Glycosidase/Glycosyl transferase a. Sugar/polymer synthesis b. Cleavage of glycosidic linkages to form mono, all-and oligosaccharides c. Synthesis of complex oligosaccharides d. Glycoside synthesis using UDP-**galactosyl transferase** e. Transglycosylation of disaccharides, glycosyl fluorides, aryl galactosides f. Glycosyl transfer in oligosaccharide synthesis g. Diastereoselective cleavage of p-glucosylsulfoxides h. Asymmetric glycosylations i. Food processing j. Paper processing

Detailed Description Paragraph Table - DETL (5):

TABLE 4 ##STR34## 4-methyl umbelliferone wherein R = G2 .beta.-D-galactose .beta.-D-glucose .beta.-D-glucuronide **GB3** .beta.-D-cellotrioside .beta.-D-cellobiopyranoside GC3 .beta.-D-galactose .alpha.-D-galactose GD3 .beta.-D-glucose .alpha.-D-glucose GE3 .beta.-D-glucoronide GI3 .beta.-D-N,N-diacetylchitobiose GJ3 .beta.-D-fucose .alpha.-L-fucose .beta.-L-fucose GK3 .beta.-D-mannose .alpha.-D-mannose non-Umbelliferyl substrates GA3 amylose [polyglucan .alpha.1,4 linkages], amylopectin [polyglucan branching .alpha.1,6 linkages] GF3 xylan [poly, 1,4-D-xylan] GG3 amylopectin, pullulan GH3 sucrose, fructofuranoside